CHRD 2024: Abstract Submission Form

Presenter Name
Ashraf Kadar Shahib

Presenter Status
Masters Student

Role in the project
Design
Perform Experiments
Analyze Data
Write Abstract

Research Category
Clinical

Title
Unravelling the Epigenetic Consequences of MeCP2 Defects in Brain Tissue in Rett Syndrome

Background
Rett Syndrome (RTT) is a neurological condition primarily affecting females and typically identified in infants by 1-2 years of age. Methyl-CpG-binding protein 2 (MECP2) gene mutations are the major cause of RTT. MeCP2 is the main methyl-binding protein in the brain. MeCP2 is a chromatin-binding nuclear protein, that binds to methylated DNA. The chromatin structure consists of an array of nucleosomes, each being made up of an octamer of core histones. The linker histone-H1 binds to the linker-DNA sequences helping in linear compaction of the chromatin. MeCP2 competes with histone-H1 binding to the linker DNA, with significant importance in neurons.

Objective
1. To investigate the impact of MeCP2 loss-of-function and non-sense mutation on the epigenomic landscape of the murine brain and how it affects gene transcription.
2. To study the impact of MeCP2 loss-of-function and non-sense mutation on the global protein translation.
3. To study the impact of MeCP2 loss-of-function and non-sense mutation on chromatin accessibility.

Methods
I employ experiments including histone and total cell protein extraction from different brain regions of RTT-associated MeCP2 mutations using male transgenic mice with knock in mutations (T158M and R255X) and their wild-type controls. Further, I perform Western Blotting against the histone proteins to analyse how the histone levels are being affected. Finally, the results of wild-type mice are compared with those of the mutant mice, to study the impact of MeCP2 mutations on histone proteins, DNMTs (DNA methyltransferases), TETs (Ten-eleven translocation family proteins), and chromatin accessibility.

Results
We have evidence suggesting that the levels of histone H1 are significantly increased between wild-type and R255X mutants in a specific brain-region. We also have a trend suggesting that the levels of histone H1 decrease in the mutants compared to the wild-types in the case of another brain-region, but increase in the case of a specific brain-region.

Conclusion
The aim of this project is to study the effects of MeCP2 loss-of-function on the global epigenome landscape. Understanding the interplay between MeCP2 and histone H1, and their relation to core histones, is critical in the brain development and neurological complications associated with Rett Syndrome. Moving forward, I aim to investigate the differences in the chromatin architecture between the wild-types and MeCP2 mutants.

Do you have a table/figure to upload?
Yes

Authors

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Ashraf Kadar Shahib	kadarsh1@myumanitoba.ca	Presenting Author	Graduate
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APPENDIX:

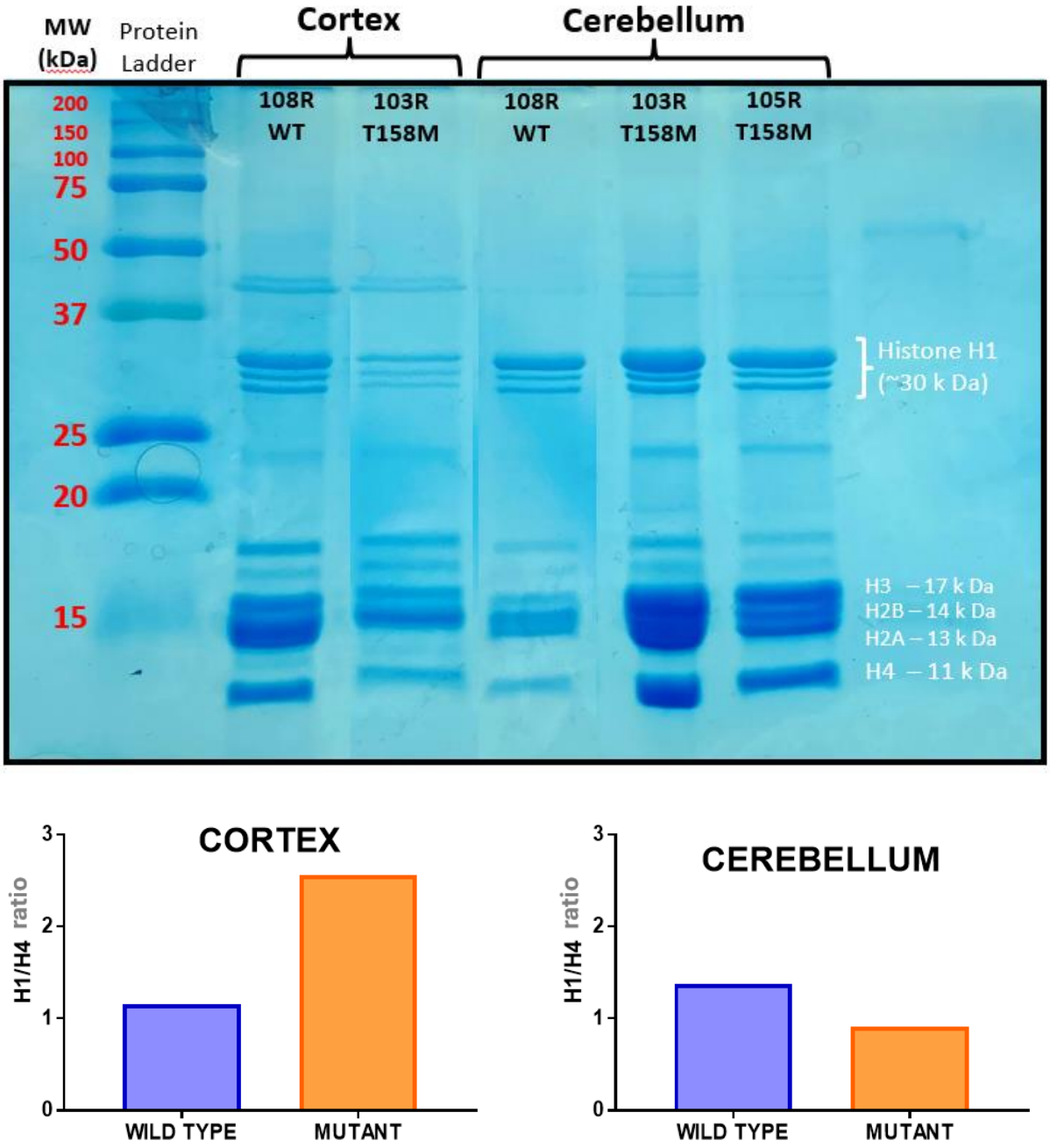


Figure 1*: Extraction (acid based) and analysis of histones [linker histone H1 and core histones (H2A, H2B, H3, and H4)] followed by separation using SDS – PAGE in the two brain regions (cerebellum and cortex) of Wildtype (108R, 160B) and T158M mutant (103R, 105R) male mice, 8 weeks old. The mice IDs and their types have been marked in the figure, accordingly.*

The maintenance of the mice colony, genotyping of the mice, and brain dissections were performed by members of Dr. Rastegar's Laboratory (Khatereh Saie Arezoumnad, Ghanan Bin Akhtar, and Carl Olson). The histone extraction protocol was developed and optimized in collaboration with Dr. James Davie's laboratory.

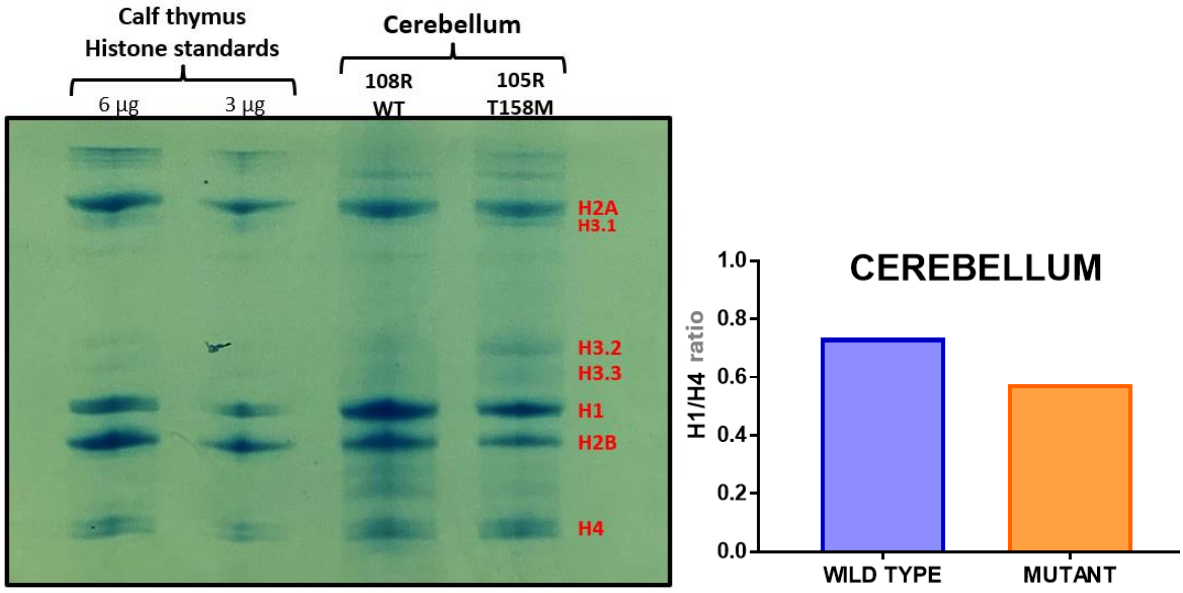


Figure 2*: Extraction (acid based) and analysis of histones [linker histone H1 and core histones (H2A, H2B, H3, and H4)] followed by separation using AUT gel in the cerebellum of Wildtype (108R) and T158M mutant (105R) male mice, 8 weeks old. The mice IDs and their types have been marked in the figure, accordingly.*

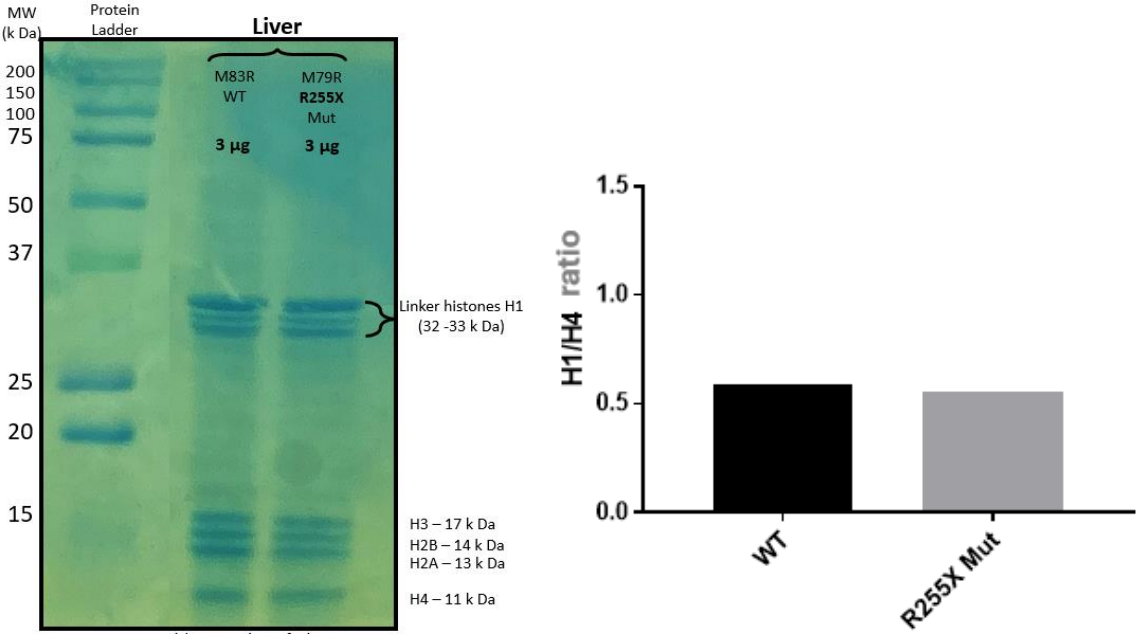


Figure 3*: Extraction (acid based) and analysis of histones [linker histone H1 and core histones (H2A, H2B, H3, and H4)] followed by separation using SDS – PAGE in the liver (as a control of my project) of Wildtype (M83R) and R255X mutant (M79R) male mice, 8 weeks old. The mice IDs and their types have been marked in the figure, accordingly.*

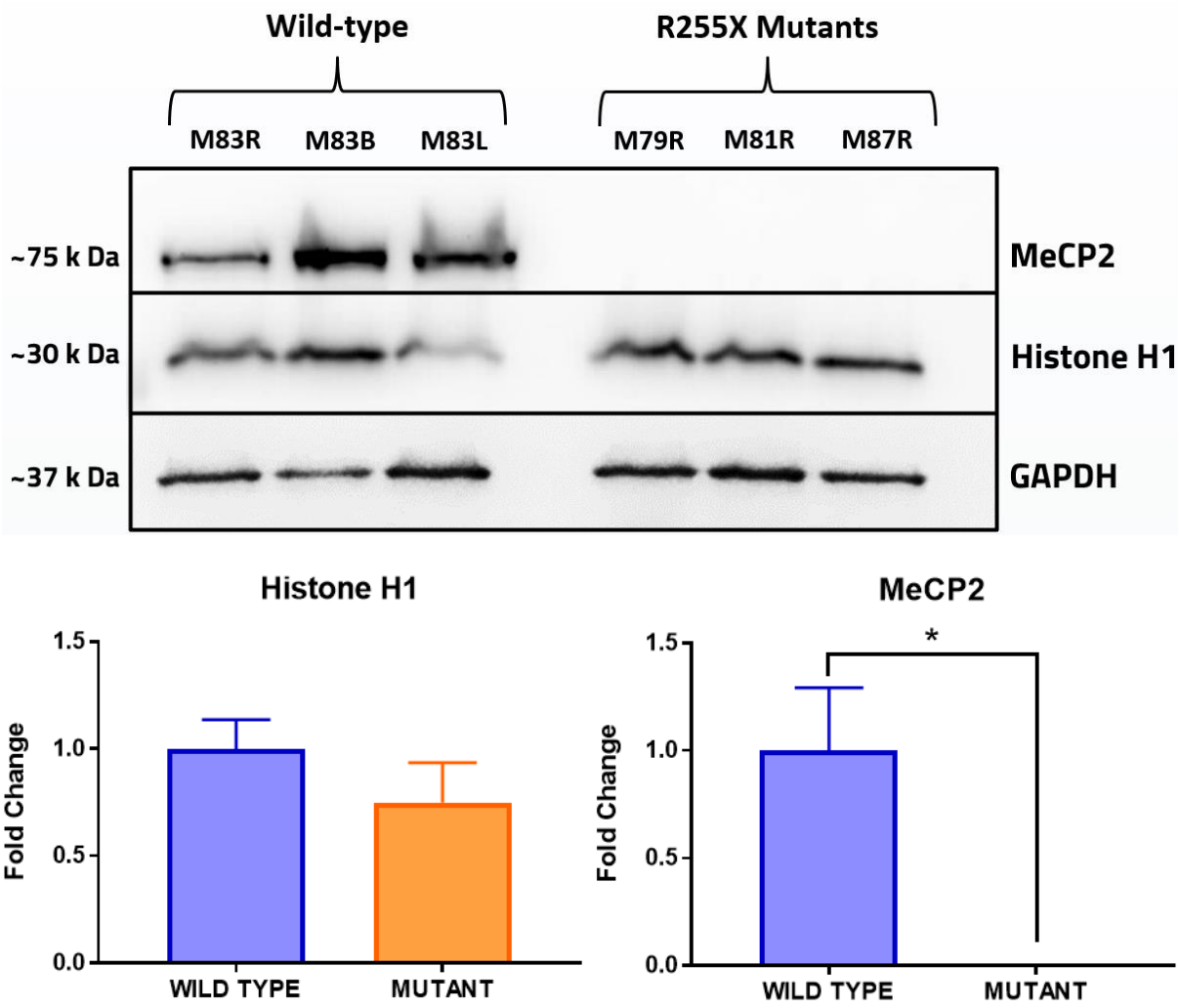
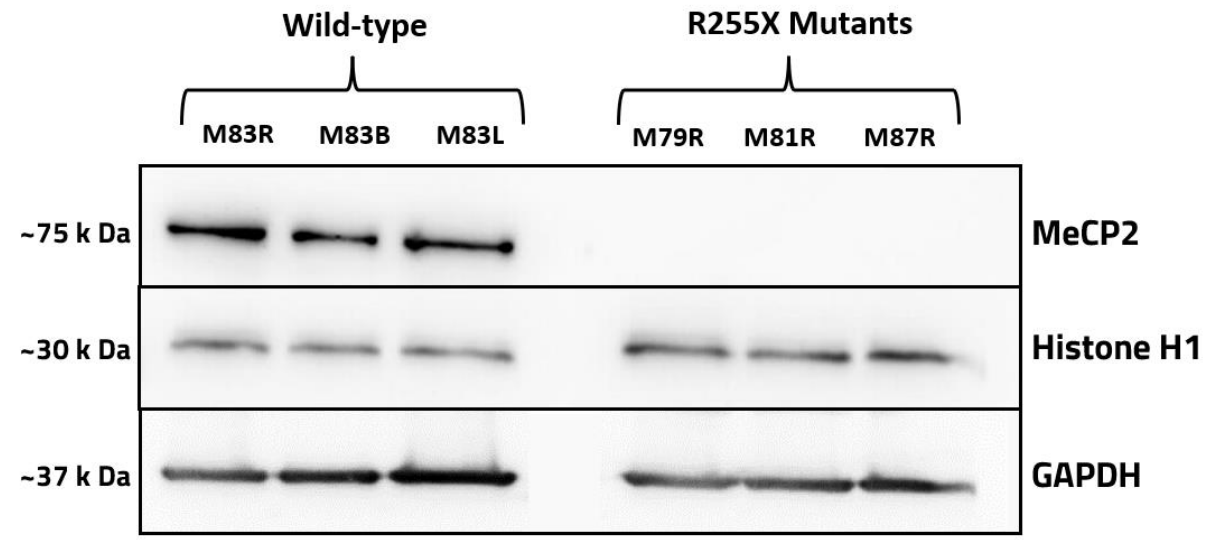


***Figure 4**: Extraction (total cell) and Western Blot Analysis of Histone H1 and MeCP2 in **cerebellum** of Wildtype (triplicates – M83R, M83B, M83L) and R255X mutant (triplicates – M79R, M81R, M87R) male mice, 8 weeks old. The mice IDs and their types have been marked in the figure, accordingly. Unpaired t test | Mean ± SEM, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.*



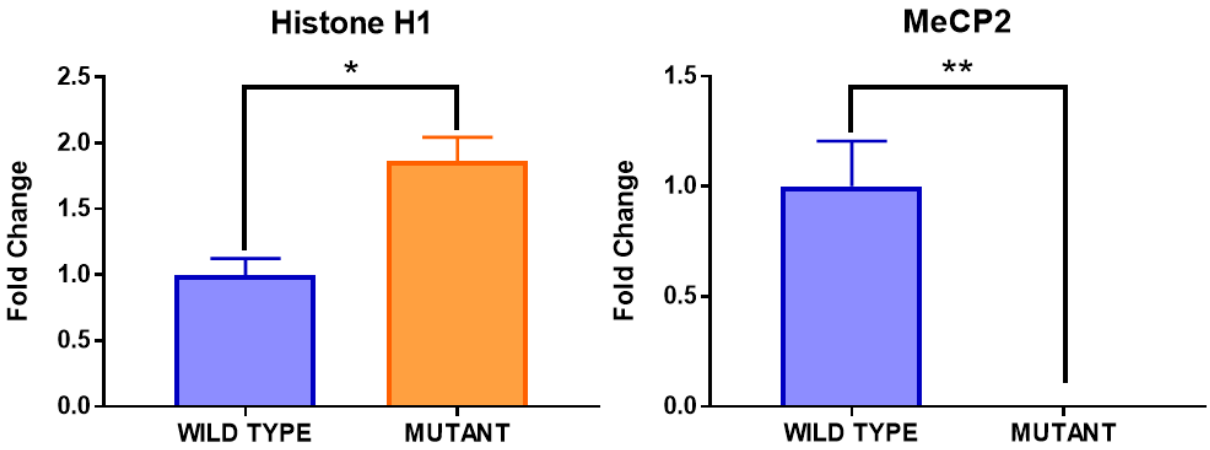


***Figure 5**: Extraction (total cell) and Western Blot Analysis of Histone H1 and MeCP2 in **hemisphere** of Wildtype (triplicates – M83R, M83B, M83L) and R255X mutant (triplicates – M79R, M81R, M87R) male mice, 8 weeks old. The mice IDs and their types have been marked in the figure, accordingly. Unpaired t test | Mean ± SEM, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.*